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Request for grant of a patent

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1.	**Your reference**	11.3.69118
2.	**Patent application number** *(The Patent Office will fill in this part)*	22 OCT 1998 9823175.6
3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Nycomed Imaging AS Nycoveien 2 P.O. Box 4220 Torshov N-0401 Oslo Norway
	Patents ADP number *(if you know it)*	
	If the applicant is a corporate body, give country/state of incorporation	Norway 62469610001
4.	**Title of the invention**	Compound
5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001

6.	**If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**	**Country**	**Priority application number** *(if you know it)*	**Date of filing** *(day / month / year)*

7.	**If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**	**Number of earlier application**	**Date of filing** *(day / month / year)*

8.	**Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	-
Description	20
Claim(s)	-
Abstract	-
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10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Frank B Dehn & Co Date 22 October 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Louise Golding
0171 206 0600

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Notes

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69118.602

Compound

This invention relates to compounds useful as contrast agents in magnetic resonance imaging and to methods of imaging using such compounds.

Magnetic resonance (MR) imaging is a well established imaging modality in which the image is derived from the intensity of the nmr signal from protons (usually water protons) in the subject under study. Because most tissue has an approximately 80% water content, contrast in MR imaging is attained by the application of pulse sequences that reveal differences in the relaxation times (T_1 and T_2) of the tissues. As with other diagnostic imaging modalities such as CT and ultrasound, contrast agents may be used in MR imaging procedures to enhance contrast in the images produced, e.g. to allow clearer differentiation between different tissue types or between healthy and non-healthy tissue. In MR imaging, the contrast agents conventionally are chelated paramagnetic species (e.g. Gd DTPA, Gd DTPA-BMA and Gd HP-DO3A, available commercially under the trade names Magnevist, Omniscan and Pro-Hance), which achieve contrast enhancement because of their relaxivities, their ability to decrease the relaxation times of water protons.

A proposal has been made, in WO96/38184, that "triggered" paramagnetic metal ion complexes be used as MR contrast agents. As described in WO96/38184, the trigger mechanism has the paramagnetic complex being "turned on" as an MR contrast agent by the presence of a target substance which interacts with the agent complexing the paramagnetic metal ion so as to free an inner sphere coordination site and allow water molecule exchange to take place at the freed-up site. In the absence of the target substance, the complexed paramagnetic metal ion has no inner sphere coordination

sites available for water molecule exchange and in this state the contrast agent is considered to be turned off.

This concept of a triggered MR contrast agent however has a major defect which will hinder practical application of the concept. Thus in the "turned off" state the complex will still function fairly effectively as an MR contrast agent since both inner-sphere and outer-sphere water coordination contributes to the agent's relaxivity. The inventors of WO96/38184 indirectly acknowledge this drawback when they refer to the degree of change in MR signal that is sufficient to be detectable in the image as being as low as 2 to 5%, well below the conventionally accepted threshold of 10% (see for example Chem. Rev. 87: 901-927 (1987)). The relaxivity of the gadolinium chelates of WO96/38184 will be reduced by about one half (but not eliminated) if inner sphere coordination of water is prevented. Thus the triggered agents of WO96/38184 are not so much switched off as dimmed by about half by the absence of the target substance. Accordingly the selectivity and sensitivity desired by the authors is not possible due to the unavoidable outer-sphere contribution.

It has since been proposed by the applicants in PCT/GB98/01173 (as yet unpublished) that triggered MR imaging of contrast agents may be achieved significantly more efficiently by using the "target substance" to change the contrast agent between states in which the relaxivity (r_1) differs by a factor of at least 5. This is achieved either by switching to a lower relaxivity state with little or no relaxivity or alternatively by switching on/off an inner sphere deriving relaxivity which is significantly higher than (e.g. 5 times or greater than) the outer sphere deriving component of the relaxivity.

Certain contrast agents which have now been found to be particularly suitable for use in "triggered" MR imaging techniques are those comprising lanthanide

compounds which can be switched between first and second oxidation states differing in relaxivity by a factor of 5 or more, preferably 10 or more, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero. "Triggered" MR imaging is achieved using such agents as a result of a redox reaction.

Thus viewed from one aspect the invention provides a method of generating a contrast enhanced image of a human or non-human (preferably mammalian) animal subject which comprises administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating an image of at least part of said subject containing said agent, wherein said agent comprises a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

In the method of the invention the change between high and low relaxivity states is effected as a change in the oxidation state of the lanthanide metal in the contrast agent between higher and lower relaxivity states. In this regard, the means for effecting the change between higher and lower relaxivity states may be localised normal or abnormal biological activity, an administered chemical agent or an applied physical means (e.g. illumination with light).

The change in oxidation state may give rise to a change in relaxivity in a number of ways, e.g. as a

result of a change from a paramagnetic to a diamagnetic state, from a diamagnetic to a paramagnetic state, or from one paramagnetic state to another. Conveniently, the change in relaxivity of the contrast agent is effected as a change from one paramagnetic state to another, e.g. from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state. The non-spherically symmetric state will have a much lower associated relaxivity than the spherically symmetric state and accordingly the contrast difference between the "on" and "off" states of the switchable agent is large.

Preferably, the contrast agent for use in the method of the invention is a chelate complex of a lanthanide metal ion in which the chelated metal ion is capable of redox conversion from one oxidation state to another (one or both of which are paramagnetic). On/off switching by a redox reaction may occur either as a result of oxidation or reduction of the chelated metal ion. Depending on the particular lanthanide metal present, its initial oxidation state and the nature of the complexing agent, this may bring about either a decrease or increase in relaxivity of the contrast agent.

Preferred contrast agents for use in the invention are those in which the "on position" corresponds to a state in which the relaxivity is as high as possible and in which the "off position" corresponds to a state in which the relaxivity is as low as possible, preferably close to zero. In this regard, contrast agents comprising Europium compounds, in particular chelate complexes of Europium, which are activated by switching between the II and III oxidation states, e.g. by biological activity or by redox reagents are

particularly preferred for use in the method of the invention.

Due to a half filled 4f shell, Eu(II) complexes have a spherically-symmetric electronic ground state ($^8S_{7/2}$) and therefore have long electron spin relaxation times and particularly high relaxivities. Eu(III) complexes, on the other hand, have a $^7F_0$ electronic ground state and very short electronic relaxation times Eu(III) is only paramagnetic because excited states must be considered, but these states are not spherically symmetric. Consequently, electronic relaxation times are very short and relaxivities are essentially zero. Oxidation of Eu(II) to Eu(III) thus causes a substantial loss of relaxivity which is readily detectable as a marked change in MR signal intensity. The transition from Eu(II) to Eu(III) thus provides a highly sensitive "on-off" switch. Moreover, the transition from Eu(II) to Eu(III) is particularly sensitive to oxygen concentration and pH:

$$Eu(II) + H^+ + \tfrac{1}{4}O_2 = Eu(III) + \tfrac{1}{2}H_2O \quad (I)$$

$$Eu(II) + H^+ = Eu(III) + \tfrac{1}{2}H_2 \quad (II)$$

Equation (I) is dominant when oxygen is present

Suitable complexing agents for use in the invention are those which present the lanthanide metal, in particular Europium, in a biotolerable form, e.g. a polyaminopolyacid chelating agent of the type well known for MR agents and radiopharmaceuticals, for example DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT, DPDP, etc. In this regard the reader is referred to the patent publications of metal chelates from Schering, Nycomed, Salutar, Bracco, Mallinckrodt, Guerbet, Sterling Winthrop, etc. Examples include US-A-4647447, US-A-5362475, US-A-5534241, US-A-5358704, US-A-5198208, US-A-4963344, EP-A-230893, EP-A-130934, EP-A-606683, EP-A-438206, EP-A-434345, WO 97/00087, WO 96/40274,

WO 96/30377, WO 96/28420, WO 96/16678, WO 96/11023, WO 95/32741, WO 95/27705, WO 95/26754, WO 95/28967, WO 95/28392, WO 95/24225, WO 95/17920, WO 95/15319, WO 95/09848, WO 94/27644, WO 94/22368, WO 94/08624, WO 93/16375, WO 93/06868, WO 92/11232, WO 92/09884, WO 92/08707, WO 91/15467, WO 91/10669, WO 91/10645, WO 91/07191, WO 91/05762, WO 90/12050, WO 90/03804, WO 89/00052, WO 89/00557, WO 88/01178, WO 86/02841 and WO 86/02005.

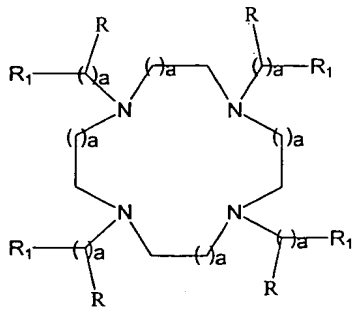
Thus appropriate complexing agents include macrocyclic chelants having an open coordination site for water, e.g. porphyrin-like molecules and the pentaaza macrocyclic ligands of Zhang et al (Inorg. Chem. 37(5):956-963, 1998), phthalocyanines, crown ethers e.g. nitrogen crown ethers such as the sepulchrates, cryptates etc., hemin (protoporphyrin IX chloride) and heme (available from Porphyrin Products, Inc. of Logan, Utah, USA) and chelants having a square-planar symmetry. Alternatively, the complexing agent may comprise a polyacid ligand capable of protonating a coordinating group thereby freeing up a coordination site for water molecules at a particular pH.

Other complexing agents of use according to the invention include polyoxadiazamacrobicyclic ligands ("cryptands") known to form stable coordination compounds ("cryptates") with several lanthanide metal ions, in particular with Europium (see J. Am Chem. Soc. 102(7): 2278-2285, 1980). In this regard, the (2.2.1), (2.2.2) and (2_β.2.1) cryptands are particularly suitable for use in the invention [the numerals within the parentheses refer to the number of oxygen atoms in the polyether bridges joining the nitrogen bridgeheads in the bicyclic molecule. Thus, (2.2.1) cryptand = 4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane and (2.2.2) cryptand = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8.8.8]hexacosane. The ligand (2_β.2.1) is similar to (2.2.1) except that one of the central

dioxyethylene groups is replaced by the analogous catechol].

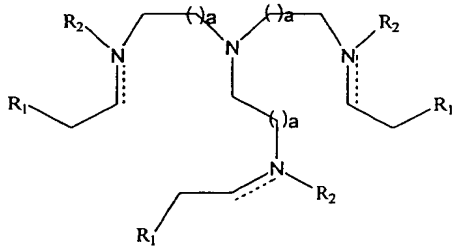
Particular Europium compounds for use in the invention include the following cryptates: $Eu^{II}(2.2.1)$, $Eu^{II}(2_{\beta}.2.1)$, $Eu^{II}(2.2.2)$ and the corresponding Eu^{III} complexes, $Eu^{III}(2.2.1)$, $Eu^{III}(2_{\beta}.2.1)$ and $Eu^{III}(2.2.2)$.

Suitable complexing agents also include ligands of formula (I)



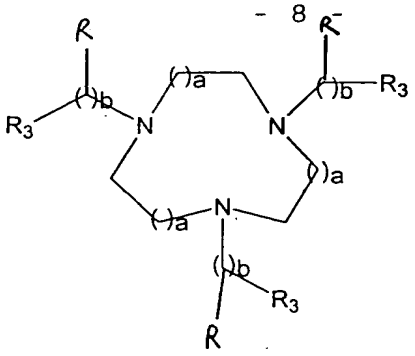
where each a independently represents an integer between 1 and 3, preferably 1, each R independently represents hydrogen or hydroxy and each R_1 independently represents a carboxylate, phosphate, thioacid, thiol, amino alkoxide or hydroxy group, preferably carboxylate;

formula (II)



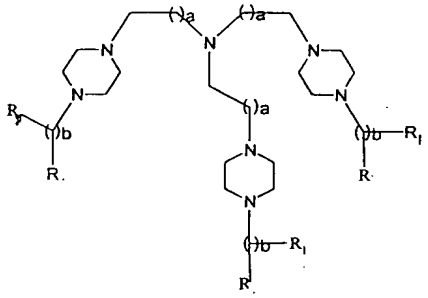
where a and R_1 are as hereinbefore defined and each R_2 independently represents hydrogen, C_{1-6} alkyl e.g. methyl or isopropyl, aryl e.g. phenyl with the proviso that R_2 is absent when the double bond is present on the same nitrogen;

formula (III)

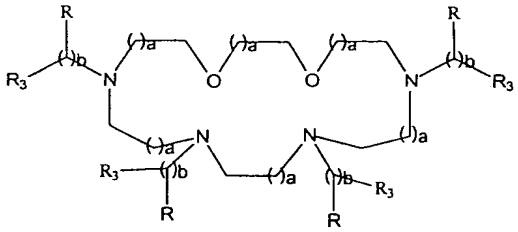


where a, R and R_2 is as hereinbefore defined, b is an integer between 0-3 and each R_3 independently represents R_1, $NR\text{-}NR_2\text{-}COO^{\ominus}$, or $N{=}N\text{-}COO^{\ominus}$ when b is positive or each R_3 independently represents $N{=}C\text{-}COO^{\ominus}$ or $NR_2\text{-}C\text{-}COO^{\ominus}$;

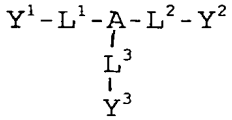
formula (IV)



where a, R and R_1 are as hereinbefore defined; and formula V



where a, b, R and R_3 are as hereinbefore defined. Also of use are complexing agents of formula (VI)



where A is N, CR_4, P, P=O, *cis,cis,cis*-1,3,5-trisubstituted-cyclohexane or an N,N',N"-trisubstituted-triaza 9 to 14 membered macrocyclic ring;

L^1, L^2, L^3 are linker grups which are independently chosen from C_{1-4} alkylene, C_{4-8} cycloalkylene or C_{4-8} o-arylene;

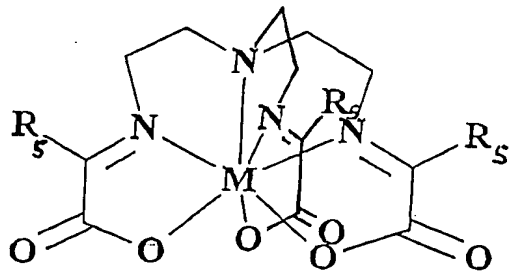
Y^1, Y^2, Y^3 are independently chosen from $-NH_2$, $-B(=O)OZ$, $-N=CR_5-B(=O)OZ$, $-NR_5-CR_6-B(=O)OZ$, $-N[CR_6-B(=O)Q]_2$ and $-O-CR_6-B(=O)OZ$ where B is C or PR_6 each Q is independently -OZ or $-NR_6$ and Z is H or a counter-ion;

each R_4 and R_5 group is independently chosen from H_1 C_{1-5} alkyl, C_{1-5} alkoxyalkyl, C_{1-5} hydroxyalkyl, C_{1-5} aminoalkyl, C_{5-10} aryl or C_{1-6} fluoroalkyl;

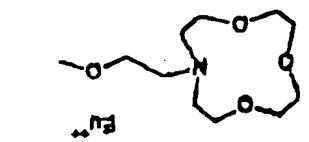
R_6 is OH, C_{1-6} alkyl, C_{1-6} alkoxyalkyl, C_{1-6} fluoroalkyl, C_{1-10} alkoxy or C_{5-10} aryl;

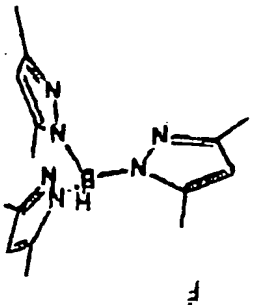
with the proviso that at least one of Y^1, Y^2 and Y^3 is $-N=CR_5-B(=O)OZ$.

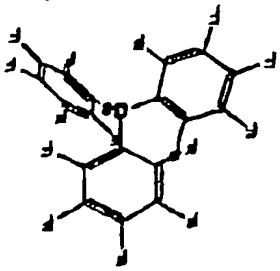
For example

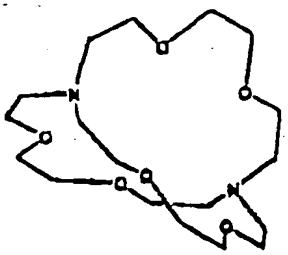


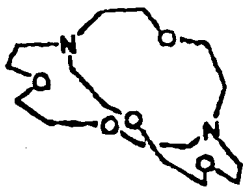
Specific complexing agents of use according to the invention include

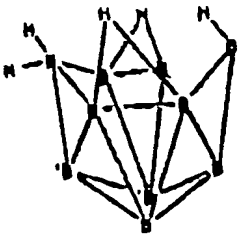


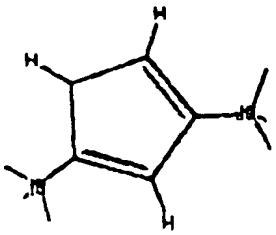


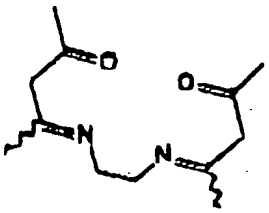


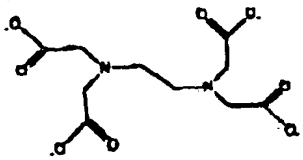


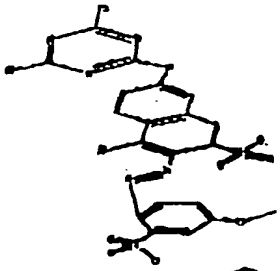


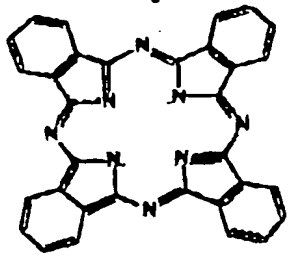


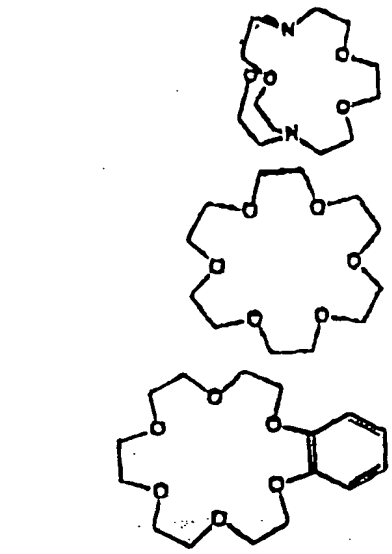


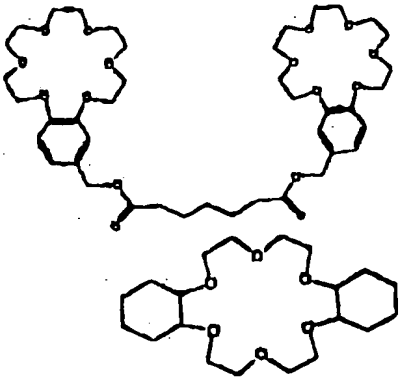


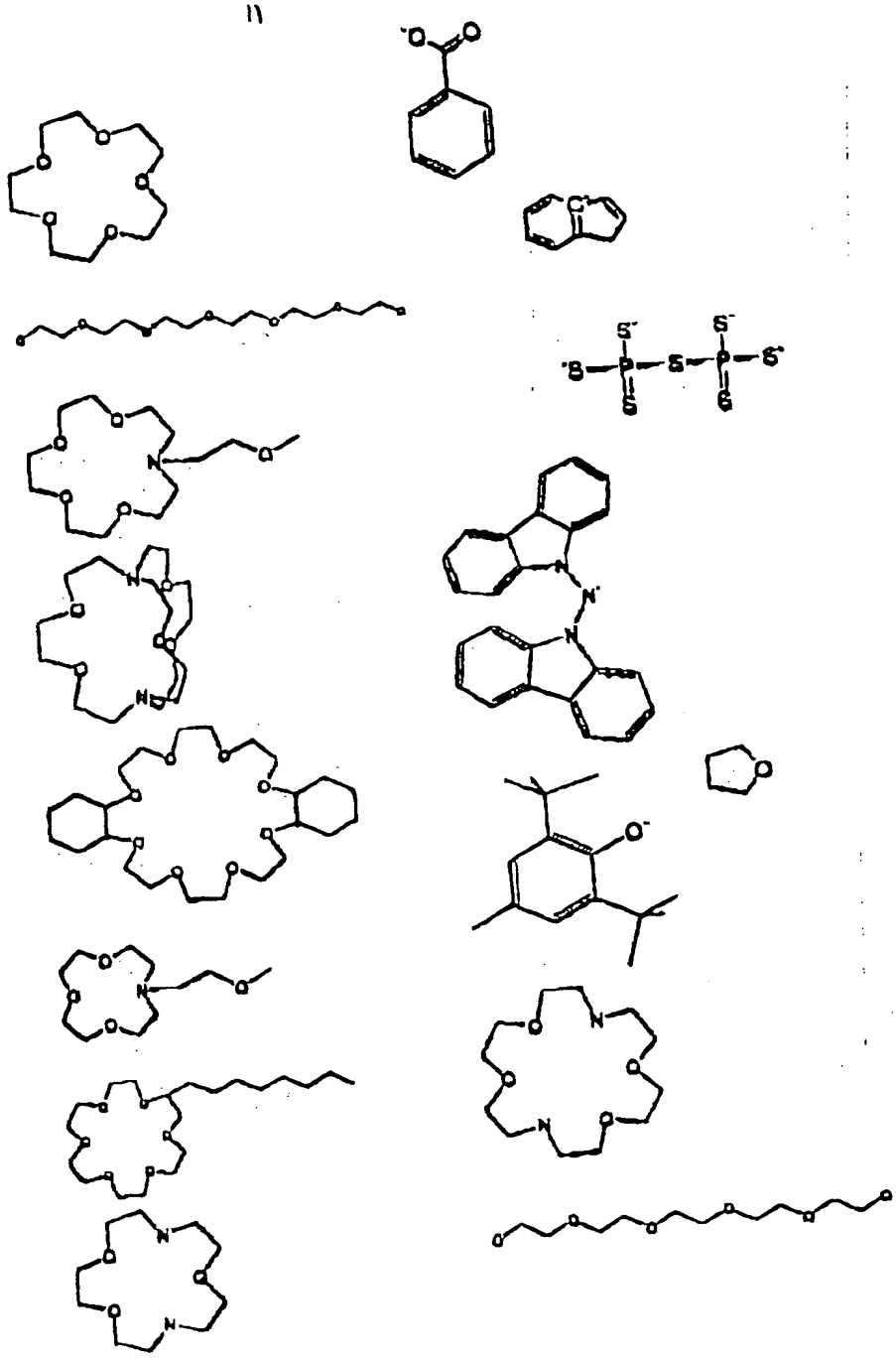












Certain complexing agents may affect the redox couple and may stabilise the metal in a higher or lower oxidation state. The complexing agent may also significantly affect the biodistribution. For example depending on the charge on the metal ion and the degree of ionisation of the complexing agent, the metal complexes of use in the present invention may be charged or neutral. Neutral complexes, which do not carry highly hydrophilic substituents may be sufficiently lipophilic to cross lipid membranes such as cell membranes or the blood-brain barrier. Lipophilicity can be readily adjusted by varying the natire of the complexing agent and will be readily achieved by the skilled person. The change in relaxivity in switching between different oxidation states may be further enhanced by having as the first oxidation state a very high relaxivity compound, such as a polymeric chelate of the lanthanide metal ion, or a rigid paramagnetic polychelate of the lanthanide metal ion, e.g. a vector targeted lanthanide chelate or polychelate, or a dendrimeric chelate of a lanthanide metal ion such as described in WO93/06868 with a short linkage or a hydrophobic linkage between dendrimeric branching sites. Further enhancement of the sensitivity of the "on-off" switch may be achieved by having as the second oxidation state a very low relaxivity compound. This may be achieved, for example, by having as the low relaxivity compound the same material with the lanthanide metal present in the second oxidation state and (i) with water coordination sites reversibly blocked by an enzymically removable blocking group (e.g. as described in WO96/38184) and/or (ii) with a targeting vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif such as RGD) which in that state is not bound to its intended substrate.

Thus the switching between low and high relaxivity states may be further enhanced by binding of the

targeting vector or displacement of the blocking groups or alternatively by interaction at the target site to change a high relaxivity conformation into a low relaxivity conformation, or *vice versa*. Such conformational changes can be achieved for the compounds of PCT/GB96/01308 by changing the chemical nature of their immediate environment (e.g. by the presence or addition of urea).

Preferably, the contrast agent for use in accordance with the invention may be further conjugated to a macromolecule. In this way the relaxivity of the contrast agent is further increased thereby enhancing the sensitivity of the "on-off" switch between relaxivity states. Examples of suitable macromolecules include proteins and polymers, e.g. that prepared in accordance with Example 2 of WO98/10797, and macrostructures such as liposomes in which the chelate is bound to the outer surface.

The means by which conversion from one oxidation state to another may be achieved may be a biological process or malfunction. Accordingly, the method of the invention finds application in methods of "functional" MR imaging capable of providing vital information relating to the functioning of particular parts of the body. For example, the method of the invention can be used to identify parts of the body which may be functioning abnormally, e.g. as a result of disease.

Transition between the "on" and "off" states may, for example, result from the presence or absence of oxygen or of oxidation or reduction promoting agents, from a change in temperature or as a result of an increase or decrease in pH at the target site, or as a result of the presence of a specific enzyme. For example, in the case of an "on-off" system, the MR image will appear bright unless there is a specific condition present such as oxygen deficiency which causes switching of the contrast agent to the lower relaxivity state and

a corresponding reduction of image intensity to that which would be expected in the absence of the contrast agent.

Alternatively the means for conversion may be a chemical agent administered to the subject, e.g. a redox reagent capable of delivery to or accumulation at a desired target site within the body, or designed for release at such a site for example a tumour or oedema. In some cases, activation of the agent may involve application of light, preferably with a wavelength of from 600 to 1300 nm in order to minimise absorption by the body.

As mentioned above, in one aspect of the invention the relaxivity of the contrast agent may be switched as a result of a change in pH. Contrast agents for use in the method of the invention may thus be used to detect areas of the body which are acidic or basic due to physiological or disease processes. Typically, they may be used to detect regions of pH of about 4 to ~5.5 within the body by appropriate selection as the contrast agent of a substance having a pKa value above or below a predictable threshold.

For example, many tumors exhibit a lower extracellular pH, e.g. as low as 5.5, typically between ~5.5 and ~7.7. This is a result of decreased vascular perfusion resulting in chronic hypoxia and increased lactic acid levels, exacerbated by the typically higher metabolic rate of cancerous cells.

Certain metal complexes undergo more rapid hydrolysis at lower pH, e.g. those of formula (VI) above as described in **WO-XXXXXX(no. please Paul)**which is herein incorporated by reference. Due to the rapid hydrolysis, metal ions are selectivley trapped in the area of low pH allowing targeting of the metal ion to certain areas of the body.

On the other hand, necrotic areas within tumors may exhibit a higher, more basic, pH. Acidic tumor types

which may be detected using the method of the invention include malignant melanoma, squamous cell carcinoma, sarcomas and adenocarcinomas (see Thistlewaite et al., Int. J. Radiation Oncology Biol. Phys. 11: 1647-1652, 1985).

Osteoporosis is a degenerative bone disorder. During the physiological process of bone resorption osteoclasts excavate small pits throughout the bone, creating a zone of reduced pH between the osteoclast and the bone tissue. pH values as low as 4.0 have been measured in the active erosion zones (see Silver et al., Cell Res. 175: 266-267, 1988). Effective imaging of this erosion zone using the method of the invention may be used to provide vital information regarding the effectiveness of therapies used in the treatment of osteoporosis. In this way, the clinician may readily determine the therapeutic effect of a given drug and use the information either to continue therapy or to change therapies.

Measurement of local osteoclastic activity using the method of the invention may also be used to evaluate other bone remodelling activities such as the repair of fractures, the treatment of Paget's disease or to evaluate the extent of expanding lesions in bone, such as tumors, in which resorption may take place at the bone surface in contact with the lesion.

In the method of the invention, the region in which the conversion from first to second relaxivity state occurs will preferably be identified, e.g. by comparison with a "native" image in the collection of which the means for conversion has not been administered or activated or with a comparison body site in which the biological process responsible for the conversion does not occur.

The contrast agents for use in the method of the invention, in particular those comprising chelating agents, may if desired be conjugated to biological

vectors so as to target actively or passively to the desired regions of the body. Conjugation of metal chelates to targeting vectors is discussed for example in WO93/21957 and US-A-5595725 (Schering).

Where the targeting vector is such as to bind the agent to a target site, relaxivity will be increased as a result and in one embodiment of the invention the triggering of enhanced relaxivity may be achieved by a combination of the freeing up of a coordination site according to WO96/38184 and binding to a larger structure, e.g. a cell wall or the wall of a body duct using a vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD) conjugated to a compound according to WO96/38184.

The contrast agent for use in the method of the invention may be for example a complex of a lanthanide metal ion having first and second oxidation states and, in the high relaxivity state, at least one open coordination site for the exchange of water molecules. Such agents are capable of switching between first and second relaxivity states as a result of a change in pH. In the case of Eu(II), a change in pH may be sufficient to alter the chelate so that this becomes very sensitive to oxygen concentration and so able to make the transition to Eu(III).

For administration into the GI tract, it may be unnecessary to chelate the metal and thus for this route simple salts, e.g. chlorides, may be used.

The contrast agent may be administered by any convenient route, eg. topical, transdermal, nasal, sub-lingual, oral, rectal, by direct instillation into an externally voiding body cavity (eg. lungs, uterus, GI tract and bladder), or subcutaneously, intramuscularly, interstitially or into the vasculature, eg. by injection or infusion. In general administration into the vasculature or into the GI tract will be preferred routes.

For administration, the contrast agent may be formulated together with appropriate conventional pharmaceutically acceptable carrier or excipients, such as liquid carriers (eg. saline or water for injections), pH and osmolality regulators, stabilizers, viscosity modifiers, surfactants, bulking agents, skin penetration agents, flavourings, solid or semi-solid carriers (eg. hydrophilic gels), aerosol dispersants, etc.

The dose of contrast agent required will depend on the species and condition under study, the selected contrast agent, and the administration route. However in general doses for i.v. administration will normally be in the range 0.001 to 5.0 mmol paramagnetic centre/kg bodyweight (where by paramagnetic centre is meant a metal atom which is or becomes paramagnetic).

If a chemical agent (a "trigger", for example an enzyme, a redox agent or a free radical scavenger) is administered to trigger the conversion between states of different relaxivity, then this can be administered together with the contrast agent or separately, eg. before or after or even simultaneously in the event that the administration site is different. If coadministered, then the trigger may be formulated to contact the contrast agent only after delivery or on reaching the target site. Thus for example it may be encapsulated in a matrix or membrane (e.g. a vesicle membrane) which breaks down or is broken down at the desired target site. If the method of the invention is used intraoperatively, e.g. to highlight damage to a particular tissue or organ or to delineate a mass to be removed, the trigger may be applied to the operating site during the operation so as to switch on the contrast agent at the cutting site. In this event the trigger may be a chemical agent as discussed above, the air, or an applied physical stimulus.

The compositions containing both the contrast agent and a chemical trigger are themselves new and form a

further aspect of the invention. Viewed from this aspect the invention provides an MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second oxidation states.

Viewed from a further aspect the invention also provides the use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

All documents referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the accompanying non-limiting Examples.

Example 1

This example involves the use of a Eu(II)-chelate for detecting a low oxygen concentration in a tumor. An

Eu(II)-chelate would be injected intravenously and a T_1-weighted pulse sequence would be used to obtain the images for a variety of times post injection of the Eu(II)-chelate. In the first image obtained at a time immediately after injection, the blood pool containing the Eu(II)-chelate would appear as being bright (high signal intensity). As time elapses, the Eu(II)-chelate will distribute into tissue, and the Eu(II) will oxidise to Eu(III) at a rate that depends on the local oxygen concentration. As a result of the oxidation, the signal intensity of images obtained at later times of regions containing the Eu-chelate will decrease as more Eu(III) is formed. The signal intensity will decrease more rapidly with time in regions of high oxygen concentration. Therefore, regions of low oxygen concentration will have a signal intensity that decreases more slowly with time, and these regions could eventually appear as bright spots on the images. Such a sensitivity to oxygen concentration could prove very useful in the characterisation of tumors, for example. The sensitivity to oxygen concentration could also prove useful in the evaluation of cardiac tissue and possibly stroke as well. However, in the evaluation of stroke and/or brain perfusion, it may be useful to use a T_2-weighted pulse sequence.

Example 2

This example involves the use of an Eu(II)-chelate conjugated to a macromolecule, designed for characterising the oxygen content of a tumor. As an example, the Eu(II)-chelate-macromolecule complexes are similar to the Gd(III)-chelate-macromolecule complexes described in T.S. Desser, K.I. Rubin, H.H. Muller, F. Qing, S. Khodar, G. Zanazzi, S.W. Young, D.L. Ladd, J.A. Wellons, K.E. Kellar, J.L. Toner, R.A. Snow, "Dynamics of Tumor Imaging with Gd-DTPa-Polyethylene Glycol

Polymers: Dependence on Molecular Weight" Journal of Magnetic Resonance Imaging 4, 467-472 (1994), where Eu(II) takes the place of Gd(III). In this work, macromolecular complexes have been shown to have an extended lifetime in the blood pool as a result of conjugating the metal chelate to a polymer, and this increased liftime enables the complexes to be taken up by tumors. However, unlike their Gd(III)-containing counterparts, the Eu(II)-containing macromolecular complexes will be sensitive to the oxygen content of the tumors as described in Example 1 above.